FORM-PTO-1390 (Rev. 12-29-99) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 003300-804

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/889229

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE00/00047	13 January 2000	14 January 1999

TITLE OF INVENTION: MOLECULARLY IMPRINTED MICROSPHERES PREPARED USING PRECIPITATION POLYMERISATION

APPLICANT(S) FOR DO/EO/US: KLAUS MOSBACH, LEI YE, and PETER A.G. CORMACK

It is contemplated that this Amendment be prosecuted while using Claims 1 to 19 that were submitted in March 7, 2001 during the international phase of the examination as further amended in the Preliminary Amendment filed herewith.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Signed Declaration will follow)
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    A copy of the International Preliminary Examination Report with Claims 1-19 submitted on March 7, 2001 is provided.

A certified copy of Swedish Application No. 9900121-6, filed 14 January 1999, was submitted during the international phase of prosecution. Thus, the claim for priority has been perfected.

JC18 Rec'd PCT/PTO 13 JUL 2001

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/889229	INTERNATIONAL APPLICATION NO PCT/SE00/00047	ATTORNEY'S DOCKET NUMBER 003300-804

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00 (960)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00 (970)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00 (958)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00 (956)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1,000.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(e)).	$ ---	

Claims	Number Filed	Number Extra	Rate		
Total Claims	20 -20 =	0	X$18.00 (966)	$ ---	
Independent Claims	1 -3 =	0	X$80.00 (964)	$ ---	
Multiple dependent claim(s) (if applicable)			+$270.00 (968)	$ ---	
TOTAL OF ABOVE CALCULATIONS =				$ 1,000.00	
Reduction for 1/2 for filing by small entity, if applicable (see below).				$ ---	-
SUBTOTAL =				$ 1,000.00	
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ ---	
TOTAL NATIONAL FEE =				$ 1,000.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +				$ ---	
TOTAL FEES ENCLOSED =				$ 1,000.00	
				Amount to be: refunded	$
				charged	$

a. ☐ Small entity status is hereby claimed.

b. ☒ A check in the amount of $ 1,000.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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(703) 836-6620

Filed: July 13, 2001

Benton S. Duffett, Jr.
SIGNATURE

Benton S. Duffett, Jr.
NAME

22,030
REGISTRATION NUMBER

Patent
Attorney's Docket No. 003300-804

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
KLAUS MOSBACH et al.	)	
	)	**BOX PCT**
Application No.: Unassigned	)	**ATTENTION: DO/EO/US**
	)	
Filed: July 13, 2001	)	
	)	
For: MOLECULARLY IMPRINTED MICROSPHERES PREPARED USING PRECIPITATION POLYMERISATION	)	Group Art Unit: Unassigned
	)	Examiner: Unassigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a national phase filing of International Application No. PCT/SE00/00047, filed January 13, 2000. It is contemplated that this Application be prosecuted in the United States while using Claims 1 to 19 that were submitted on March 7, 2001 during the international phase of examination as further amended herein.

Please amend this Application as indicated.

IN THE ABSTRACT:

Please add the Abstract if the Disclosure that is provided on a separate sheet.

IN THE CLAIMS:

Kindly replace Claims 1, 3, 4, 7, 10, and 12 to 19 as follows:

1. (Amended) A method of producing molecularly imprinted microspheres comprising specific binding sites, comprising polymerising functional monomers and crosslinkers in a reaction solvent in the presence of print molecules as templates in a surfactant-free precipitation polymerisation process, which print molecules are capable are capable of forming non-covalent or reversible covalent interactions with said functional monomers.

3. (Amended) A method according to claim 1, wherein the reaction solvent is aqueous or non-aqueous.

4. (Amended) A method according to claim 1, wherein said reaction solvent is composed of a single solvent component or of multiple solvent components.

7. (Amended) A method according to claim 1, wherein the solubility of the print molecules in the reaction solvent is adjusted by changing the composition of the reaction solvent.

10. (Amended) A method according to claim 1, wherein a desired size of the microspheres is achieved by controlling the nucleation and particle growth process.

12. (Amended) A method according to claim 10, wherein the control of the nucleation and particle growth process is intended to avoid aggregation of the microspheres.

13. (Amended) A method according to claim 1, wherein the size of the microspheres as produced is in the range of 0.01-10μm.

14. (Amended) A method according to claim 1, wherein the reaction conditions are controlled so that the microspheres become monodisperse.

15. (Amended) A method for screening of chemical libraries, for catalysis, for facilitating synthesis, for analyte determination using ligand binding assays and/or agglutination assays, for therapeutic purposes, or for controlled release comprising using the molecularly imprinted microspheres according to claim 1.

16. (Amended) A method for conducting capillary electrophoresis, capillary electrochromatography or HPLC analysis comprising using the molecularly imprinted microspheres according to claim 1 as the stationary phase or as a modifier.

17. (Amended) A biomimetic sensor comprising the molecularly imprinted microspheres according to claim 1 as a recognition component.

18. (Amended) An affinity-labelled probe for targeting cells or other biological material comprising the molecularly imprinted microspheres according to claim 1.

19. (Amended) A composite material comprising the molecularly imprinted microspheres according to claim 1 as a binding entity.

Please add the following new Claim 20:

20. (New) A method according to claim 1, wherein the reaction solvent is aqueous or non-aqueous.

REMARKS

The present amendment modifies the claim format and eliminates the use if multiple dependency.

The examination and allowance of the Application are respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____

Benton S. Duffett, Jr.
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P.O. Box 1404
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Date: July 13, 2001

Attachment to Preliminary Amendment dated July 13, 2001

Marked-up Claims 1, 3, 4, 7, 10, and 12 to 19

1. (Amended) A method of producing molecularly imprinted microspheres comprising specific binding sites, [characterised by] comprising polymerising functional monomers and crosslinkers in a reaction solvent in the presence of print molecules as templates in a surfactant-free precipitation polymerisation process, which print molecules are capable are capable of forming non-covalent or reversible covalent interactions with said functional monomers.

3. (Amended) A method according to claim 1 [or 2], wherein the reaction solvent is aqueous or non-aqueous.

4. (Amended) A method according to claim 1 [or 1], wherein said reaction solvent is composed of a single solvent component or of multiple solvent components.

7. (Amended) A method according to claim 1 [or 2], wherein the solubility of the print molecules in the reaction solvent is adjusted by changing the composition of the reaction solvent.

10. (Amended) A method according to claim 1 [or 2], wherein a desired size of the microspheres is achieved by controlling the nucleation and particle growth process.

Attachment to Preliminary Amendment dated July 13, 2001

Marked-up Claims 1, 3, 4, 7, 10, and 12 to 19

12. (Amended) A method according to claim 10, wherein the control of the nucleation and particle growth process is [such as] intended to avoid aggregation of the microspheres.

13. (Amended) A method according to claim 1 [or 2], wherein the size of the microspheres as produced is in the range of 0.01-10μm.

14. (Amended) A method according to claim 1 [or 2], wherein the reaction conditions are controlled so that the microspheres become monodisperse.

15. (Amended) [Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14,] A method for screening of chemical libraries, for catalysis, for facilitating synthesis, for analyte determination using ligand binding assays and/or agglutination assays, for therapeutic purposes, or for controlled release comprising using the molecularly imprinted microspheres according to claim 1.

16. (Amended) [Use of the molecularly imprinted microspheres as prepared according to any one if claims 1-14, as stationary phase or modifier in] A method for conducting capillary electrophoresis, capillary electrochromatography or HPLC analysis

Attachment to Preliminary Amendment dated July 13, 2001

Marked-up Claims 1, 3, 4, 7, 10, and 12 to 19

comprising using the molecularly imprinted microspheres according to claim 1 as the stationary phase or as a modifier.

17. (Amended) [Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14, as recognition component in] A biomimetic [sensors] sensor comprising the molecularly imprinted microspheres according to claim 1 as a recognition component.

18. (Amended) [Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14, as] An affinity-labelled probe for targeting cells or other biological material comprising the molecularly imprinted microspheres according to claim 1.

19. (Amended) [Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14, as binding entities for the preparation of] A composite [materials] material comprising the molecularly imprinted microspheres according to claim 1 as a binding entity.

Abstract of the Disclosure

Molecularly imprinted microspheres comprising specific binding site are described. These microspheres can be obtained by a method comprising polymerising functional monomers and crosslinkers in a reaction solvent in the presence of print molecules as templates in a surfactant-free precipitation polymerisation process. The print molecules used are capable of forming non-covalent, reversible covalent or semi-covalent interactions with said functional monomers. There is also disclosed the use of said microspheres in different applications.

Patent

Attorney's Docket No. 003300-804

Applicant or Patentee: Klaus Mosbach et al.

Application or Patent No.: 09/889,229

Filed or Issued: July 13, 2001

For: MOLECULARLY IMPRINTED MICROSPHERES PREPARED USING PRECIPITATION POLYMERIZATION

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§ 1.9(f) AND 1.27(b)) - INDEPENDENT INVENTOR

As a below-named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 C.F.R. § 1.9(c) for purposes of paying reduced fees under Sections 41(a) and 41(b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled "Molecularly imprinted microspheres prepared using precipitation polymerisation" described in:

[ ] the specification filed herewith
[X] Application No. _____, filed 13 July 2001
[ ] Patent No. _____, issued _____.

I have not assigned, granted, conveyed, or licensed and am under no obligation under contract or law to assign, grant, convey, or license any rights in the invention either to any person who could not be classified as an independent inventor under 37 C.F.R. § 1.9(c) if that person had made the invention, or to any concern that would not qualify as either a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[X] no such person, concern, or organization
[ ] persons, concerns, or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27.)

FULL NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

FULL NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

FULL NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earlier of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b).)

Application Serial No. 09/889,229
Attorney's Docket No. 003300-804

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name Klaus Mosbach

Signature Klaus Mosbach Date aug. 3, 2001

Name Lei Ye

Signature Lei Ye Date August 21, 2001

Name Peter A.G. Cormack

Signature see separate Declaration Date

Patent

Attorney's Docket No. 003300-804

Applicant or Patentee: Klaus Mosbach et al.

Application or Patent No.: 09/889,229

Filed or Issued: July 13, 2001

For: MOLECULARLY IMPRINTED MICROSPHERES PREPARED USING PRECIPITATION POLYMERIZATION

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 C.F.R. §§ 1.9(f) AND 1.27(b)) - INDEPENDENT INVENTOR

As a below-named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 C.F.R. § 1.9(c) for purposes of paying reduced fees under Sections 41(a) and 41(b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled "Molecularly imprinted microspheres prepared using precipitation polymerisation" described in:

[ ] the specification filed herewith
[X] Application No. _____, filed 13 July 2001
[ ] Patent No. _____, issued _____.

I have not assigned, granted, conveyed, or licensed and am under no obligation under contract or law to assign, grant, convey, or license any rights in the invention either to any person who could not be classified as an independent inventor under 37 C.F.R. § 1.9(c) if that person had made the invention, or to any concern that would not qualify as either a small business concern under 37 C.F.R. § 1.9(d) or a nonprofit organization under 37 C.F.R. § 1.9(e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

[X] no such person, concern, or organization
[ ] persons, concerns, or organizations listed below*

*NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. § 1.27.)

FULL NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

FULL NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

FULL NAME _____

ADDRESS _____

[ ] individual [ ] small business concern [ ] nonprofit organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earlier of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. § 1.28(b).)

Application Serial No. 09/889,229
Attorney's Docket No. 003300-804

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name Klaus Mosbach

Signature see separate Declaration Date _____

Name Lei Ye

Signature see separate Declaration Date _____

Name Peter A.G. Cormack

Signature Peter Cormack Date 30/8/1

MOLECULARLY IMPRINTED MICROSPHERES PREPARED USING PRECIPITATION POLYMERISATION

The present invention relates to molecularly imprinted microsperes, to a method of producing said microspheres and to the use of said microspheres.

More particularly, the present invention relates to a method for preparing molecularly imprinted microspheres in the absence of any added surfactants. Highly specific, molecularly imprinted microspheres in the micron-scale size range can be produced quickly, cleanly and in excellent yield by this method, and the regular particle size and shape of the microspheres obtained is advantageous in several ways. These artificial receptors can readily replace biologically derived receptors in many applications and are therefore highly attractive. Several possible applications are described herein.

BACKGROUND OF THE INVENTION

Molecular imprinting is an established technique for the preparation of synthetic receptors with high affinities and specificities for various analytes of interest. During the free-radical polymerisation commonly used in the imprinting process, the incorporation of template-complementary functionality into the polymer matrix, which is the key to ligand re-binding, is guided by the template molecules themselves, since they form complementary guest-host complexes with the functional monomers. Following removal of the template from the polymer matrix, the crosslinked polymeric host can rebind the original template very specifically (Figure 1) [1-3].

Depending on the nature of the interactions guiding the assembly of the guest-host complex and the subsequent recognition of the target ligand, molecular imprinting strategies can be divided into two major categories: covalent and non-covalent imprinting approaches. A semi-

covalent imprinting method is also reported, where one can use covalent interactions for the preparation of the imprinted polymer and non-covalent interactions for the subsequent re-binding of ligands of interest [4].

These molecularly imprinted receptor analogs are easy to produce and very stable, and are therefore superior to natural receptors in many respects.

Molecularly imprinted polymers have been used for chromatographic separation [5], in biomimetic sensors [6], in catalyzing chemical reactions [7], in solid phase extraction for sample enrichment/clean-up [8], in screening of combinatorial chemical libraries [9], for *in situ* product removal during biotransformation processes [10], and down-stream product purification [11]. They also have great potential for drug determination using for instance ligand competition assays [12].

Imprinted polymers are usually prepared in the form of a monolith which is then ground and sieved to the desired particle size. The grinding and sieving process is time-consuming and yields only moderate amounts of useful imprinted polymer. The polymer particles obtained are also irregularly-shaped, which is not ideal for chromatographic purposes. Furthermore, the grinding process may also be detrimental to some of the binding sites.

Suspension polymerisation in perfluorocarbon liquid continuous phases has been introduced to address some of these issues [13]. Although this method delivers good yields of spherical particles with controlled particle sizes, it is not a straightforward method in that it requires considerable optimisation. The perfluorocarbon dispersing phase is also somewhat expensive.

Other imprinting methods leading to spherical particles with controlled sizes include emulsion polymerisation in aqueous media, and seeded emulsion polymerisation. However, they involve either the use of

stabilisers or multi-step operations, which are neither straightforward nor broadly applicable for imprinting.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a new method of producing molecularly imprinted microspheres. Typically, the diameters of the microspheres are between 0.01 - 10 μm. The method is based on surfactant-free precipitation polymerisation. The specific binding sites are created using print molecules that form either non-covalent, reversible covalent or "semi-covalent" interactions with the functional monomers as templates.

In the method the total volume of the polymerisable monomer/crosslinker is typically kept within 0.01 - 20% v/v that of the reaction solvent. The reaction solvent employed is either aqueous or non-aqueous, and is either single component or composed of multiple solvents.

The interactions between print molecules and functional monomers utilised for imprinting and re-binding can be either reversible covalent, non-covalent, or both. Multiple interactions of different characters can be simultaneously utilised. Different functional monomers can be employed simultaneously, in addition to using single functional monomers.

The solubility of the print molecules in the reaction solvent can be adjusted by changing the composition of the reaction solvent.

The polymerisation can be induced by heat, by UV, by γ radiation or by chemical methods. Free-radical polymerisation, ionic polymerisation, coordination polymerisation, step growth polymerisation or related methods are used to prepare molecularly imprinted microspheres without using surfactant.

Microspheres with desired particle sizes can be produced by controlling the nucleation and particle growth process of the resulting polymer. This is achieved through adjusting the composition of functional monomer/crosslinker/solvent system, as well as reaction

conditions, in order to change the solubility of the growing polymer chains. The polymerisation conditions are controlled in such a way as to avoid aggregation of the microspheres.

The molecularly imprinted microspheres can be used as replacements for conventionally imprinted polymers in various applications. Thus, they can be used for the screening of chemical libraries, for catalysis, for facilitated synthesis, for analyte determination using competitive ligand binding assays and agglutination assays.

The microspheres can also be used as stationary phase or modifier in capillary electrophoresis, capillary electrochromatography and HPLC analysis, as recognition component in biomimetic sensors, as affinity-labelled probe for targeting cells or other biological materials.

A further use of the molecularly imprinted microspheres is as binding entities for the preparation of composite materials.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a reaction scheme of a prior art molecular imprinting process.

Figure 2 shows some examples of functional monomers which can be used in the process according to the invention.

Figure 3 shows electron micrographs of anti-17β-estradiol microspheres prepared according to Example 3.

Figure 4 shows the displacement of radioligand binding to molecularly imprinted microspheres under equilibirium conditions, as disclosed in Example 4. B/B_0 is the ratio of the amount of radioligand bound in the presence of displacing ligand, B, to the amount bound in the absence of displacing ligand, B_0.

Figure 5 shows a calibration curve for theophylline, as disclosed in Example 5.

Figure 6 shows the specificity of the anti-17β-estradiol microspheres prepared according to Example 3.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a novel method for preparing molecularly imprinted microspheres using precipitation polymerisation, the microspheres as obtained by said method, and the applications of these imprinted microspheres.

Precipitation polymerisation, sometimes called surfactant-free polymerisation, can be used to prepare mono-disperse microspheres with controlled particle diameters typically within the 0.1-10 μm range [14-17]. The mechanism for particle formation and growth resembles that of dispersion polymerisation, except that the particles are stabilised against coagulation by their rigid, crosslinked surfaces, rather than by any added stabilisers. These microspheres are easy to prepare and are free from any adsorbed surfactants. Significantly, neither polymer grinding nor sieving steps are necessary following polymerisation, therefore the preparation of molecularly imprinted microspheres by this method is much more efficient in terms of yield and much less time-consuming to perform.

In conventional molecular imprinting protocols, the yield of imprinted polymer with the desired particle size range following successive grinding and sieving operations is usually less than 50%. In contrast, the present method allows polymer yields upwards of 85% to be attained. The regular size and shape of the particles can facilitate system homogenisation and is advantageous for mass transfer in ligand rebinding processes. It also offers benefits in chromatographic applications.

Both reversible covalent and non-covalent interactions can be utilised during the imprinting process when using precipitation polymerisation. The semi-covalent strategy can also be used. Functional monomers for reversible covalent interactions include boronate ester-forming monomers, Schiff base-forming monomers, and carbonate-forming monomers. For non-covalent inter-

actions, hydrogen bond-forming monomers, ion-pair forming monomers, metal-chelating monomers, as well as hydrophobic monomers can be used (Figure 2).

Various crosslinkers can be used depending on the solvent employed as a porogen.

Polymerisation can be initiated in a variety of ways, typically via thermal or photochemical means, and both water-soluble and organic solvent miscible initiators can be used, depending on the solvents employed.

To obtain spherical microspheres with good recognition behaviour, efficient crosslinking has to be ensured. Typically this is achieved by using a high degree of crosslinking. For some purposes, however, a much lower crosslinking density can still yield microspheres with satisfactory molecular recognition capabilities.

Compared to conventional imprinting methods, far greater amounts of solvent are used in precipitation polymerisation protocols to prepare the imprinted microspheres. Both aqueous and non-aqueous solvents can be used for different target print molecules. When the non-covalent strategy is used, except where the hydrophobic effect is of interest, less-polar organic solvents, for example dichloromethane and acetonitrile are generally most satisfactory. Imprinting via other strategies can readily use aqueous and polar non-aqueous solvents. The total monomer volume in the polymerization solution is generally within the range of 1 - 10% v/v with respect to the polymerisation solvent, to prevent aggregation of the microspheres.

The amount of print molecule can be, though not necessarily, so high as to saturate the solvent containing the functional monomer and the crosslinker at the polymerisation temperature in order to provide a high load capacity for the resulting imprinted microspheres.

On the other hand, a poor solvent for the print molecule can be introduced as a co-solvent, if required, to reduce the solubility of the print molecule and therefore to reduce the amount of print molecule required. This may be an attractive approach when one is using an expensive print molecule, for example, hexane can be added to acetonitrile to make the print molecule much less soluble while the complex formation between the functional monomer and the print molecule in the non-covalent approach is not sacrificed.

By controlling various reaction conditions, such as the solubility parameters of the resulting polymer and that of the solvent, the nucleation and growth behaviour of the polymer particles can be tailored to deliver microspheres of controlled particle diameter and porosity that retain high affinity and specificity for the print molecules.

The molecularly imprinted microspheres can be used in various applications. These artificial receptors can readily replace their natural counterparts in many instances. Their regular size and shape allows better reproducibility in different assays. Non-limiting examples of applications of molecularly imprinted microspheres, including monodisperse microspheres, are: 1) as stationary phases or modifiers in capillary electrophoresis; 2) as recognition components in biomimetic sensors; 3) as catalysts to facilitate chemical/biochemical reactions; 4) as probes for cell or other biological material targeting in which case they are dyed or made magnetic; 5) for drug determination using competitive ligand assay; 6) as bio-compatible carrier for controlled drug release; 7) as binding components to prepare composite materials for affinity purification/isolation of target compounds.

The invention will now be described more in detail by way of the following non-limiting examples.

EXAMPLE 1

Preparation of anti-theophylline microspheres

Acetonitrile (50 mL) is mixed with methacrylic acid (MAA, 372.5 mg) and trimethylolpropane trimethacrylate (TRIM, 627.5 mg) in a borosilicate glass tube. Theophylline (115 mg) is suspended in the solution and dissolved after sonication at 60°C. The initiator, azobisisobutyronitrile (AIBN, 17.5 mg) is dissolved, the solution purged with nitrogen for five minutes and the tube sealed under nitrogen. Polymerisation is induced by placing the tube in a water bath preset at 60°C and continued for 24 hours.

The microspheres formed are collected by centrifugation at 8000 rpm for 10 minutes using a RC5C superspeed refrigerated centrifuge from BECKMAN (Palo Alto, CA, USA). The print molecule is thoroughly extracted by washing repeatedly with methanol containing 10% acetic acid (v/v), followed by a final wash in acetone. These successive centrifugation and decanting steps extract the print molecule from the polymer. The anti-theophylline microspheres obtained are monodisperse and have an average diameter of 0.2 μm. The microspheres are finally dried *in vacuo*. The reference (control) microspheres are prepared and treated in exactly the same way, except that no print molecule is used in the polymerisation stage.

EXAMPLE 2

Preparation of anti-theophylline microspheres

Acetonitrile (50 mL) is mixed with MAA (372.5 mg) and TRIM (627.5 mg) in a borosilicate glass tube. Theophylline (11.5 mg) and AIBN (17.5 mg) are dissolved in the solution. The solution is purged with nitrogen for five minutes and the tube sealed under nitrogen. Polymerisation is induced by UV irradiation (350 nm) at 20°C using a RMA-400 Rayonet photochemical reactor from Southern New England Ultraviolet Co. (Bradford, CT, USA) and continued for 24 hours.

The microspheres obtained are treated in the same way as in example 1 to remove the print molecule. The reference (control) microspheres are prepared and treated in exactly the same way, except that no print molecule is used in the polymerisation stage.

EXAMPLE 3

Preparation of anti-17β-estradiol microspheres

Acetonitrile (50 mL) is mixed with MAA (372.5 mg) and TRIM (627.5 mg) in a borosilicate glass tube. 17β-Estradiol (250 mg) and AIBN (17.5 mg) are dissolved in the above solution. The solution is purged with nitrogen for five minutes and the tube sealed under nitrogen. Polymerisation is induced by UV irradiation (350 nm) at 20°C using a RMA-400 Rayonet photochemical reactor from Southern New England Ultraviolet Co. (Bradford, CT, USA) and continued for 24 hours.

The microspheres obtained are treated in the same way as per example 1 to remove the print molecule. The anti-17β-estradiol microspheres obtained are monodisperse and have an average diameter of 0.3 μm (Figure 3). The reference (control) microspheres are prepared and treated in exactly the same way, except that no print molecule is used in the polymerization stage.

EXAMPLE 4

Competitive radioligand assay using anti-theophylline microspheres from example 1

The binding capacity of the anti-theophylline microspheres from example 1 is estimated from saturation studies. Varying amounts of the microspheres are incubated overnight and at room temperature with 16.2 pmol (685 Bq) [8-^{3}H]theophylline in 1 mL acetonitrile, using polypropylene microcentrifuge tubes. A rocking table ensured gentle mixing.

The microspheres are then separated by centrifugation at 14,000 rpm for five minutes, 500 μL supernatant mixed with 10 mL scintillation liquid, Ecoscint O (National Diagnostics, Manville, NJ, USA), and

the radioactivity then measured using a model 2119 RACKBETA β-radiation counter from LKB Wallac (Sollentuna, Sweden). The amount of anti-theophylline microspheres required to bind half of the added radioligand is estimated to be 5 mg, while an equivalent amount of the reference polymer binds less than 10% of the added radioligand.

The theophylline-imprinted microspheres are suspended in acetonitrile (25 mg/mL) and sonicated to form a polymer stock suspension, from which 200 µL was transferred into each microcentrifuge tube. Varying amounts of non-radiolabelled ligand, including theophylline, theobromine, xanthine and caffeine, and 16.2 pmol (685 Bq) [8-^{3}H]theophylline are added, and the final volume adjusted to 1 mL with acetonitrile. The competitive binding is allowed to proceed overnight by incubation at ambient temperature, using a rocking table for gentle mixing. The amount of bound radioligand is estimated by measuring the radioactivity from 500 µL supernatant following centrifugation at 14,000 rpm for five minutes. The high specificity of the anti-theophylline microspheres can be evaluated by comparing the IC_{50} value of compounds closely related to the print molecule, with IC_{50} being the ligand concentration that can displace 50% of the bound radioligand from the imprinted microspheres. Figure 4 shows the displacement of radioligand binding to molecularly imprinted microspheres under equilibrium condition. B/B_0 is the ratio of the amount of radioligand bound in the presence of displacing ligand, B, to the amount bound in the absence of displacing ligand, B_0.

EXAMPLE 5

Competitive radioligand assay using anti-theophylline microspheres from example 2

The same procedure as used in example 4 is followed, except that the microspheres are from example 2. The amount of anti-theophylline microspheres required to bind

half of the added radioligand is estimated to be 10 mg, while an equivalent amount of the reference polymer binds less than 20% of the added radioligand.

The theophylline-imprinted microspheres are suspended in acetonitrile (50 mg/mL) and sonicated to form a polymer stock suspension, from which 200 µL was transferred into each microcentrifuge tube. The same competitive binding assay as in example 4, using theophylline as the cold ligand, is followed. A calibration curve for theophylline similar to the one in Figure 4 is obtained, although the non-specific binding is slightly higher (Figure 5).

EXAMPLE 6

Competitive radioligand assay using anti-17β-estradiol microspheres from example 3

Varying amounts of the microspheres were incubated overnight and at room temperature with 417 fmol (1110 Bq) [2,4,6,7-^{3}H(N)]estradiol in 1 mL acetonitrile, using polypropylene microcentrifuge tubes. Other conditions are the same as used in example 4. The amount of anti-17β-estradiol microspheres required to bind half of the added radioligand is estimated to be 30 mg, while an equivalent amount of the reference polymer binds less than 12% of the added radioligand.

The 17β-estradiol-imprinted microspheres are suspended in acetonitrile (150 mg/mL) and sonicated to form a polymer stock suspension, from which 200 µL was transferred into each microcentrifuge tube. Varying amounts of non-radiolabelled ligand, including 17β-estradiol, 17α-estradiol and 17α-ethynylestradiol, and 417 fmol (1110 Bq) [2,4,6,7-^{3}H(N)]estradiol are added, and the final volume adjusted to 1 mL with acetonitrile. Other conditions are the same as used in example 4. The specificity of the anti-17β-estradiol microspheres is signified in Figure 6.

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(8) Sellergren, B. *Anal. Chem.* 1994, *66*, 1578-1582.

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AMENDED CLAIMS

1. A method of producing molecularly imprinted microspheres comprising specific binding sites, c h a r a c t e r i s e d by polymerising functional monomers and crosslinkers in a reaction solvent in the presence of print molecules as templates in a surfactant-free precipitation polymerisation process, which print molecules are capable of forming non-covalent or reversible covalent interactions with said functional monomers.

2. A method according to claim 1, wherein the total volume of polymerisable monomers and crosslinkers is kept in the range of about 0.01 to 20 % of the volume of the reaction solvent.

3. A method according to claim 1 or 2, wherein the reaction solvent is aqueous or non-aqueous.

4. A method according to claim 1 or 1, wherein said reaction solvent is composed of a single solvent component or of multiple solvent components.

5. A method according to claim 1, wherein said functional monomers have the same functionality.

6. A method according to claim 1, wherein said functional monomers have different functionality.

7. A method according to claim 1 or 2, wherein the solubility of the print molecules in the reaction solvent is adjusted by changing the composition of the reaction solvent.

8. A method according to claim 1, wherein the polymerisation is induced by heat, UV radiation, γ radiation and/or chemically.

9. A method according to claim 1, wherein said polymerisation process is a free-radical polymerisation process, an ionic polymerisation process, a coordination polymerisation process or a step growth polymerisation process.

10. A method according to claim 1 or 2, wherein a desired size of the microspheres is achieved by controlling the nucleation and particle growth process.

11. A method according to claim 10, wherein the control of the nucleation and particle growth process is achieved by adjusting the composition of the functional monomer/crosslinker/solvent system and/or the reaction conditions during the polymerisation in order to change the solubility of the growing polymer chains.

12. A method according to claim 10, wherein the control of the nucleation and particle growth process is such as to avoid aggregation of the microspheres.

13. A method according to claim 1 or 2, wherein the size of the microspheres as produced is in the range of 0.01-10μm.

14. A method according to claim 1 or 2, wherein the reaction conditions are controlled so that the microsperes become monodisperse.

15. Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14, for screening of chemical libraries, for catalysis, for facilitating synthesis, for analyte determination using ligand binding assays and/or agglutination assays, for therapeutic purposes, or for controlled release.

16. Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14, as stationary phase or modifier in capillary electrophoresis, capillary electrochromatography or HPLC analysis.

17. Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14, as recognition component in biomimetic sensors.

18. Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14, as affinity-labelled probe for targeting cells or other biological material.

19. Use of the molecularly imprinted microspheres as prepared according to any one of claims 1-14, as binding entities for the preparation of composite materials.

5

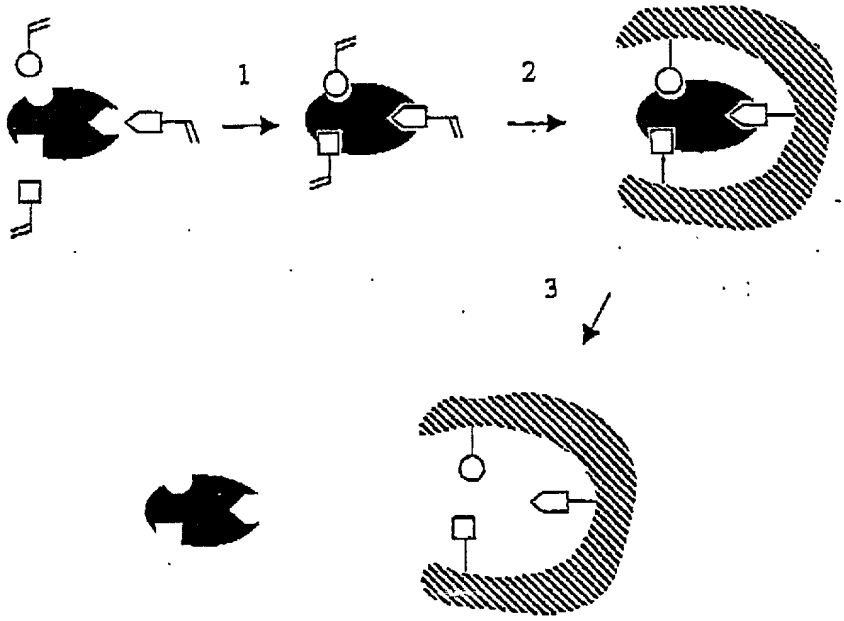
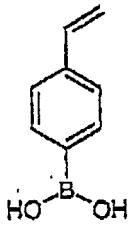


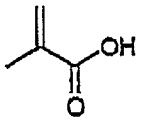
FIGURE 1. *Schematic representation of the molecular imprinting process.*
(1) Pre-assembly (2) Polymerization (3) Extraction/cleavage

Boronate ester-forming monomer

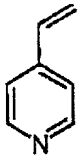


4-Vinylphenylboronic acid

Hydrogen bond-forming and ion-pair forming monomers

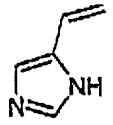


Methacrylic acid

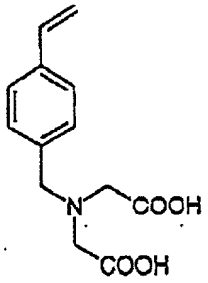


4-Vinylpyridine

Metal-chelating monomers

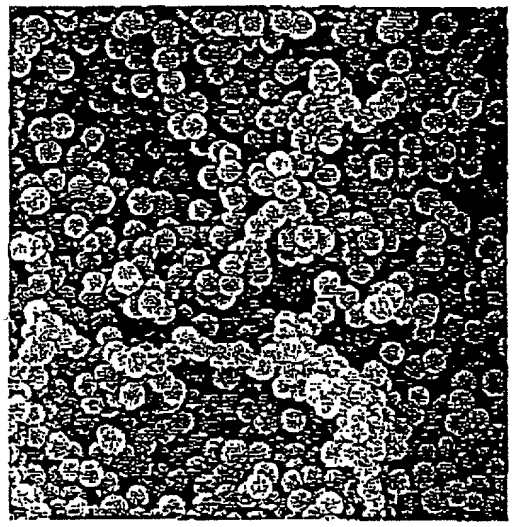


4-Vinyl imidazole



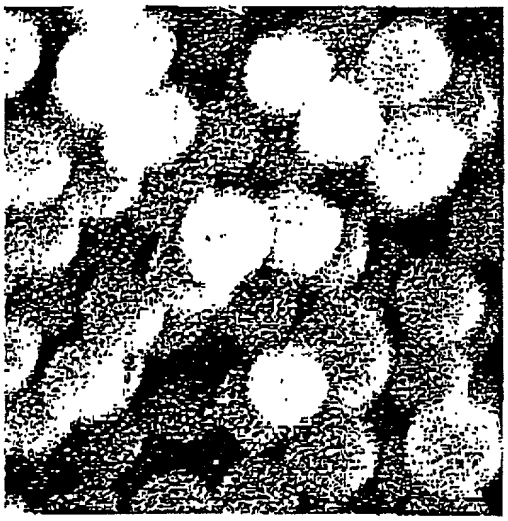
4-Vinylbenzyl-iminoacetic acid

FIGURE 2



Magnification 7,500 x

FIGURE 3a



Magnification 30,000 x

FIGURE 3b

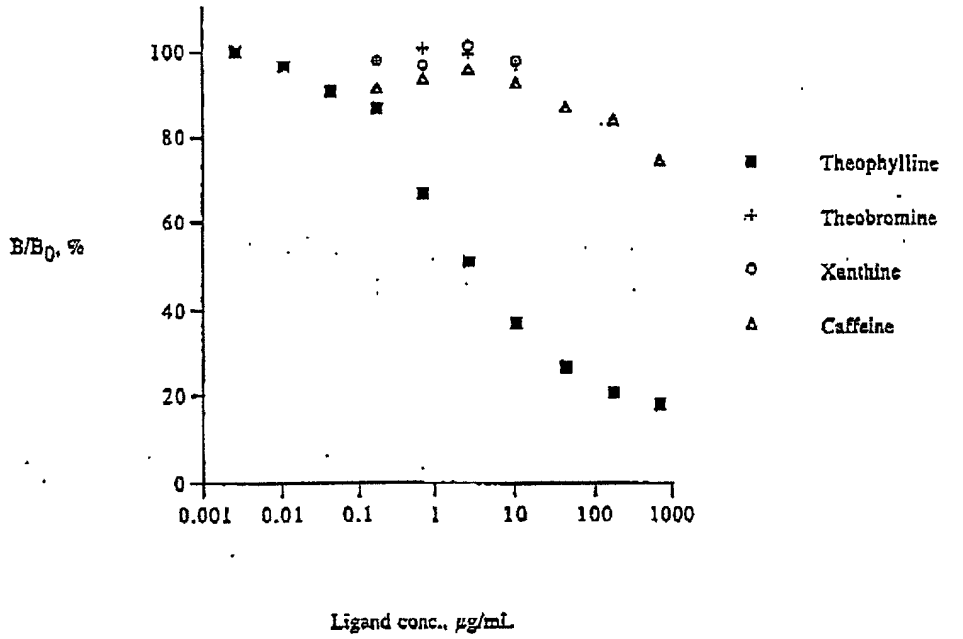


FIGURE 4

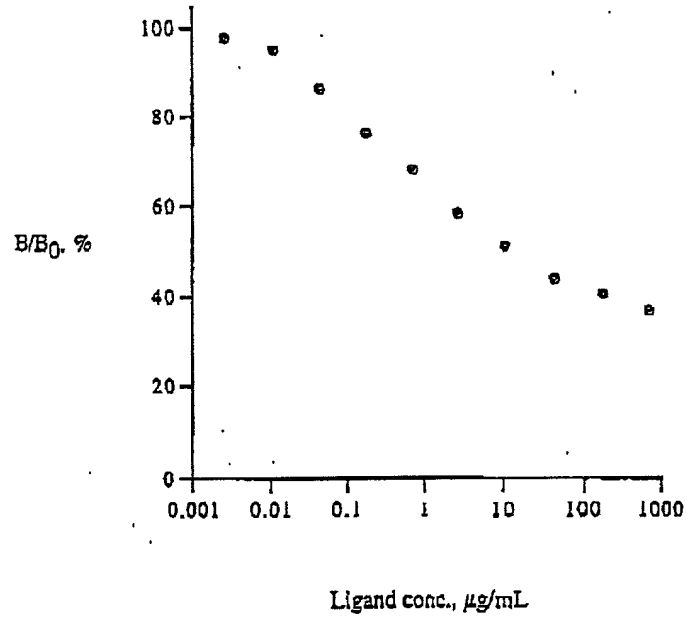


FIGURE 5

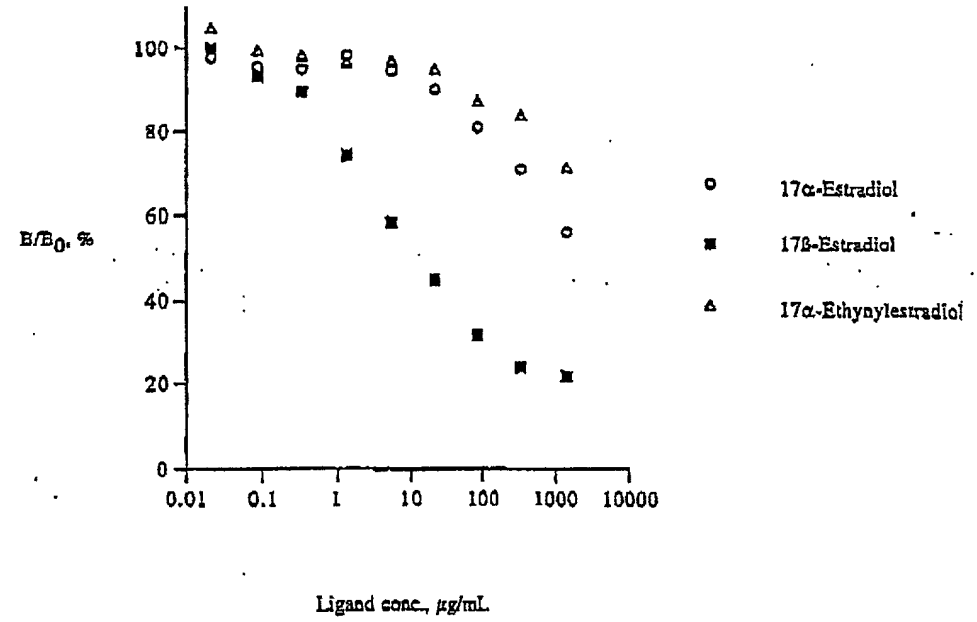


FIGURE 6

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-804

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"Molecularly imprinted microspheres prepared using precipitation polymerisation"

the specification of which (check only one item below):

☐ is attached hereto.

☒ was filed as United States application

Number _____

on 13 July 2001

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/SE00/00047

on 13 January 2000

and was amended

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Sweden	9900121-6	14 January 1999	X Yes _No
			_Yes _No
			_Yes _No
			_Yes _No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

_____ (Application Number) _____ (Filing Date)

_____ (Application Number) _____ (Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-804

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

William L. Mathis	17,337	R. Danny Huntington	27,903	Gerald F. Swiss	30,113
Robert S. Swecker	19,885	Eric H. Weisblatt	30,505	Michael J. Ure	33,089
Platon N. Mandros	22,124	James W. Peterson	26,057	Charles F. Wieland III	33,096
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Alan E. Kopecki	25,813	Patrick C. Keane	32,858	Allen R. Baum	36,086
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Robert G. Mukai	28,531	Peter K. Skiff	31,917		
George A. Hovanec, Jr.	28,223	Richard J. McGrath	29,195		
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21839

and: None

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NO. 003300-804

FULL NAME OF SOLE OR FIRST INVENTOR	SIGNATURE	DATE
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FULL NAME OF THIRD JOINT INVENTOR, IF ANY	SIGNATURE	DATE
Peter A.G. Cormack	see separate Declaration	

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FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE

RESIDENCE	CITIZENSHIP

POST OFFICE ADDRESS

FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE

RESIDENCE	CITIZENSHIP

POST OFFICE ADDRESS

FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE

RESIDENCE	CITIZENSHIP

POST OFFICE ADDRESS

FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE

RESIDENCE	CITIZENSHIP

POST OFFICE ADDRESS

FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE

RESIDENCE	CITIZENSHIP

POST OFFICE ADDRESS

FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE

RESIDENCE	CITIZENSHIP

POST OFFICE ADDRESS

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-804

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"Molecularly imprinted microspheres prepared using precipitation polymerisation"

the specification of which (check only one item below):

☐ is attached hereto.

☒ was filed as United States application

Number _____

on 13 July 2001

and was amended

on _____ (if applicable).

☒ was filed as PCT international application

Number PCT/SE00/00047

on 13 January 2000

and was amended

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Sweden	9900121-6	14 January 1999	X Yes _No
			_Yes _No
			_Yes _No
			_Yes _No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

_____ (Application Number) _____ (Filing Date)

_____ (Application Number) _____ (Filing Date)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-804

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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Platon N. Mandros	22,124	James W. Peterson	26,057	Charles F. Wieland III	33,096
Benton S. Duffett, Jr.	22,030	Teresa Stanek Rea	30,427	Bruce T. Wieder	33,815
Norman H. Stepno	22,716	Robert E. Krebs	25,885	Todd R. Walters	34,040
Ronald L. Grudziecki	24,970	William C. Rowland	30,888	Ronni S. Jillions	31,979
Frederick G. Michaud, Jr.	26,003	T. Gene Dillahunty	25,423	Harold R. Brown III	36,341
Alan E. Kopecki	25,813	Patrick C. Keane	32,858	Allen R. Baum	36,086
Regis E. Slutter	26,999	Bruce J. Boggs, Jr.	32,344	Steven M. du Bois	35,023
Samuel C. Miller, III	27,360	William H. Benz	25,952	Brian P. O'Shaughnessy	32,747
Robert G. Mukai	28,531	Peter K. Skiff	31,917		
George A. Hovanec, Jr.	28,223	Richard J. McGrath	29,195		
James A. LaBarre	28,632	Matthew L. Schneider	32,814		
E. Joseph Gess	28,510	Michael G. Savage	32,596		

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and: None

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONTINUED) (Includes Reference to Provisional and PCT International Applications)	ATTORNEY'S DOCKET NO. 003300-804

FULL NAME OF SOLE OR FIRST INVENTOR: Klaus Mosbach	SIGNATURE: see separate Declaration	DATE:
RESIDENCE: FURULUND, SWEDEN	CITIZENSHIP: SWEDEN	
POST OFFICE ADDRESS: Lackalänga 31-38, 244 94 FURULUND, SWEDEN		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY: Lei Ye	SIGNATURE: see separate Declaration	DATE:
RESIDENCE: LUND, SWEDEN	CITIZENSHIP: GREAT BRITAIN	
POST OFFICE ADDRESS: Kämnärsvägen 5D:218, 226 46 LUND, SWEDEN		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY: Peter A.G. Cormack	SIGNATURE: Peter Cormack	DATE: 30/8/1
RESIDENCE: GLASGOW G42 9TG, GREAT BRITAIN GBN	CITIZENSHIP: GREAT BRITAIN	
POST OFFICE ADDRESS: 56 Cartside Street, Flat 2/L, Langside, GLASGOW G42 9TG, GREAT BRITAIN		
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		